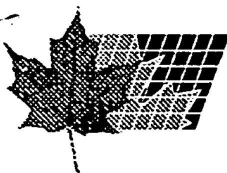

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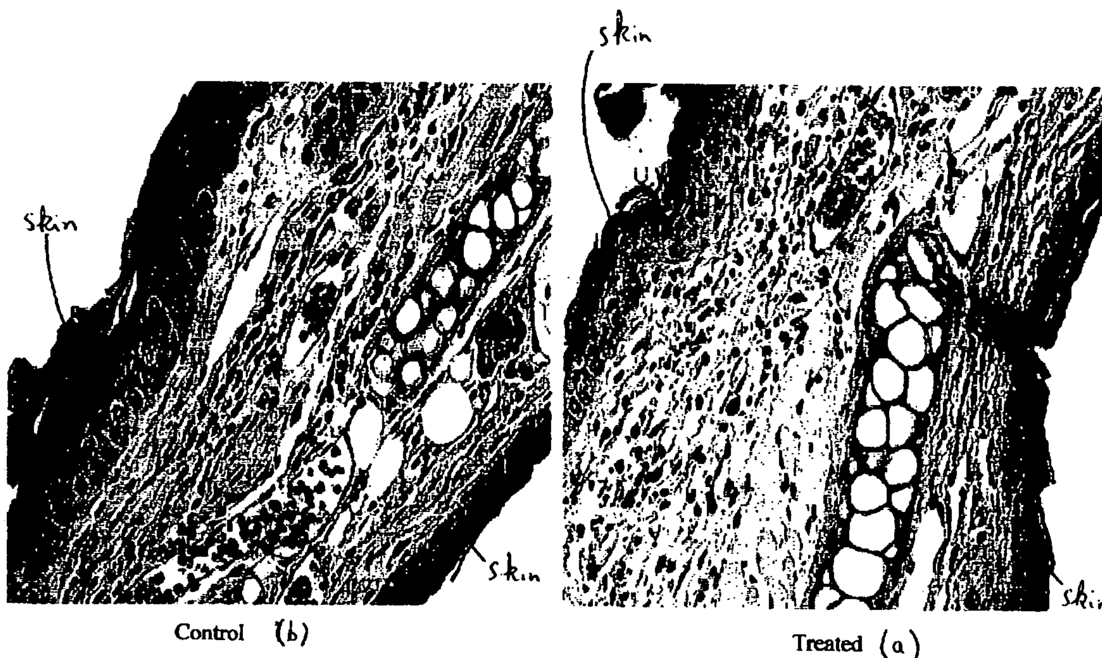
(54) **TRAITEMENT DE MALADIES INFLAMMATOIRES ET ALLERGIQUES**

(54) **TREATMENT OF INFLAMMATORY AND ALLERGIC DISORDERS**



Control (b) Treated (a)

(57) Conditions of chronic and acute inflammation and/or allergy in mammalian patients are alleviated by a process in which an aliquot of blood is withdrawn from the patient, treated extracorporeally with a combination of stressors selected from heat, UV radiation and an oxidative environment, such as an oxygen/ozone gas mixture bubbled through the aliquot, and then re-injected into the patient.

ABSTRACT OF THE DISCLOSURE

Conditions of chronic and acute inflammation and/or allergy in mammalian patients are alleviated by a process in which an aliquot of blood is withdrawn from the patient, treated extracorporeally with a combination of stressors selectod from heat, UV radiation and an oxidative environment, such as an oxygen/ozone gas mixture bubbled through the aliquot, and then re-injected into the patient.

TREATMENT OF INFLAMMATORY AND ALLERGIC DISORDERS

FIELD OF THE INVENTION

This invention relates to the field of medicine and medical treatments. In particular, the invention relates to improved methods of treating inflammatory disorders in mammalian patients, by introduction into the patient of a small amount of treated, modified mammalian blood.

BACKGROUND OF THE INVENTION

Inflammation, as the term is used in the present specification, is a localized protective response elicited by injury or destruction of tissues, which serves to destroy, dilute or wall off (sequester) both the injurious agent and the injured tissue. It is characterized in the acute form by the classical signs of pain, heat, redness, swelling and loss of function. Histologically, it involves a complex series of events, including dilatation of arterioles, capillaries and venules, with increased permeability and blood flow; exudation of fluids;, including plasma proteins; and leukocytic migration into the inflammatory focus (Dorland's Illustrated Medical Dictionary, 28th Edition, W.B.Saunders Company). Acute inflammation, which is progressive and usually of sudden onset, is characterized by the classical signs, in which the vascular and exudative processes predominate. Chronic inflammation is of slow progress and is marked chiefly by the formation of new connective tissue; it may be a continuation of an acute form or a prolonged low-grade form, and usually causes permanent tissue damage. The present invention relates to prophylaxis or treatment of the symptoms of inflammatory disorders causing either or both of

acute inflammation or chronic inflammation..

Thus the inflammatory disorder can be one involving allergic reactions of the skin or other body organs to external agents. Specific examples of such conditions include contact and delayed type hypersensitivity reactions, in which the skin of the patient exhibits an allergic reaction to an agent which the body has previously encountered, by contact or by inoculation. The "poison ivy" inflammatory allergic reaction is a specific example of contact hypersensitivity. The external agents can be plant, animal, insect or reptilian secretions, chemical or biochemical irritants, from synthetic or natural sources. Various types of fibers, fabrics and the like, such as latex used in surgical gloves, can give rise to inflammatory and/or allergic reactions such as contact hypersensitivity, in certain individuals. The offending external agents can be air-borne agents such as dusts and pollens. They can be water-borne agents such as dissolved salts and minerals. Eczema or atopic dermatitis is another example of inflammatory or allergic conditions within the scope of the present application. Inflammatory and allergic dermatological conditions of this type are to be distinguished from psoriasis, which is an autoimmune disorder which manifests itself in red scaly skin patches having an inflammatory component, but not resulting from allergic contact reaction.

Other types of inflammatory and/or allergic conditions to which the present invention is addressed are food allergies and respiratory disorders. Respiratory disorders are characterized by inflammation of the breathing tubes and lungs of the body, and include asthma and hay fever. These commonly result from allergic reactions of breathing tubes and/or their mucosal linings to external, air-borne agents inhaled by the patient. There is a known correlation between contact dermatitis and asthma - Kim, K.T., and Safadi, G.S., Relation of latex-specific

IgE titer and symptoms in patients allergic to latex. J.Allergy Clin. Immunol. 103: 671-677 (1999).

Chronic or acute inflammatory conditions are also exemplified by conditions in which the body gives an inflammation response to foreign agents introduced into the body internally. These include adverse reactions to ingested food, to pharmaceuticals, to viral or bacterial infections and their residues or secretions, and other agents. These can manifest themselves in inflammation of internal body organs as well as inflammation of the patient's skin. Examples of these are chronic inflammation of the endothelium and other components of the vascular wall associated with or resulting from atherosclerosis (which has recently been proposed to be, in some cases, at least partly the result of a chronic infectious process, chlamydia bacteria infection being the prime suspect), and adjuvant induced arthritis, where the arthritis may be due to the presence of an invasive factor, such as a residue of an infectious organism, in the joint area, e.g. in the synovial fluid or in the synovial membrane. The process of the invention addresses these and closely related inflammatory conditions also.

Mammalian blood modified by exposure simultaneously to certain stressors has been reported to be useful for the treatment of a variety of pathological conditions. The stressors to which the blood is exposed are an oxidative environment namely ozone/oxygen gas mixtures applied to the blood, a temperature stressor and UV light. Thus:

U.S. Patent No. 4,968,483 Mueller et al. describes an apparatus for oxygenating blood by treating an aliquot of a patient's blood extracorporeally, with an oxygen/ozone mixture and

ultraviolet light, at a controlled temperature. The apparatus taught by Mueller is proposed for use in hematological oxidation therapy.

U.S. Patent No. 5,591,457 Bolton discloses a method of inhibiting the aggregation of blood platelets in a human, a method of stimulating the immune system and a method of treating peripheral vascular diseases such as Raynaud's disease, by extracting an aliquot of blood from a patient, subjecting it to an ozone/oxygen gas mixture and ultraviolet radiation at a temperature in the range of about 37 to 43°C, and then re-injecting the treated blood in the human patient.

U.S. Patent No. 5,834,030 Bolton describes a process for increasing the content of nitric oxide in the blood of a mammalian subject, potentially useful in treating conditions such as high blood pressure in mammalian subjects, by subjecting a sample of the patient's blood extracorporeally to three stressors simultaneously, namely an ozone/oxygen gas mixture bubbled through the blood sample, exposure to UV radiation and an elevated temperature, followed by re-injection of the treated blood sample into the patient.

International Publication No. WO 98/07436 describes an autoimmune vaccine for administration to human patients to alleviate the symptoms of autoimmune diseases such as rheumatoid arthritis. The vaccine comprises an aliquot of the subject's blood which has been subjected extracorporeally to an oxidizing environment, UV radiation and elevated temperature, simultaneously.

International Publication No. WO 96/34613 relates to treatment of vascular disorders associated with deficient endothelial function, in a mammalian subject, by administration to the patient of an aliquot of blood which has been modified by having been subjected simultaneously to stressors namely elevated temperature in the range of 37° to 55°C, ultraviolet radiation and an oxidative environment

SUMMARY OF THE INVENTION

According to one aspect, the present invention provides a method of treatment or prophylaxis of acute or chronic inflammatory and/or allergic disorders in a mammalian patient, which comprises administering modified mammalian blood to the patient, the blood having been modified extracorporeally by simultaneous or sequential exposure to at least one stressor selected from the group consisting of an oxidative environment, UV radiation, and temperature above, at or below body temperature.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying FIGURES are presentations of the results of specific Examples described below.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

According to a preferred process of the present invention, an aliquot of blood is extracted from a mammalian subject, preferably a human, and the aliquot of blood is treated ex vivo, simultaneously or sequentially, with at least one of the aforementioned stressors. Preferably a combination of at least two or three of the aforementioned stressors is used. The stressor combination can be: (a) an oxidative environment in combination with temperature above, at or below body temperature; (b) an oxidative environment in combination with ultraviolet light; (c) a temperature above, at or below body temperature in combination with ultraviolet light; or (d) an oxidative environment in combination with both ultraviolet light and a temperature above, at or below body temperature.

Preferably also, the aliquot of blood is in addition subjected to mechanical stress. Such mechanical stress is suitably that applied to the aliquot of blood by extraction of the blood aliquot through a conventional blood extraction needle, or a substantially equivalent mechanical stress, applied shortly before the other chosen stressors are applied to the blood aliquot. This mechanical stress may be supplemented by the mechanical stress exerted on the blood aliquot by bubbling gases through it, such as ozone/oxygen mixtures, as described below.

The terms "aliquot", "aliquot of blood" or similar terms used herein include whole blood, separated cellular fractions of the blood including platelets, separated non-cellular fractions of the blood including plasma, plasma components and combinations thereof. Preferably, in human patients, the volume of the aliquot is up to about 400 ml, preferably from about 0.1 to about 100 ml, more preferably from about 1 to about 15 ml, even more preferably from about 8 to about 12 ml, and most preferably about 10 ml. The effect of the combination of stressors is to modify the blood, and/or the cellular or non-cellular fractions thereof, contained in

the aliquot. The modified aliquot is then re-introduced into the subject's body by any suitable method, most preferably intramuscular injection, but also including subcutaneous injection, intraperitoneal injection, intra-arterial injection, intravenous injection and oral administration.

The stressors to which the aliquot of blood is subjected ex vivo, optionally in combinations of two or three of such stressors according to the present invention, are selected from temperature stress (blood temperature above or below body temperature), an oxidative environment, and ultraviolet light, individually or in any combination, simultaneously or sequentially.

The temperature stressor either warms the aliquot being treated to a temperature above normal body temperature or cools the aliquot below normal body temperature. The temperature is selected so that the temperature stressor does not cause excessive hemolysis in the blood contained in the aliquot and so that, when the treated aliquot is injected into a subject, the desired effect will be achieved. Preferably, the temperature stressor is applied so that the temperature of all or a part of the aliquot is up to about 55°C, and more preferably in the range of from about -5°C to about 55°C.

In some preferred embodiments of the invention, the temperature of the aliquot is raised above normal body temperature, such that the mean temperature of the aliquot does not exceed a temperature of about 55°C, more preferably from about 40°C to about 50°C, even more

preferably from about 40°C to about 44°C, and most preferably about 42.5 ± 1°C.

In other preferred embodiments, the aliquot is cooled below normal body temperature such that the mean temperature of the aliquot is within the range of from about 4°C to about 36.5°C, more preferably from about 10°C to about 30°C, and even more preferably from about 15°C to about 25°C

The oxidative environment stressor can be the application to the aliquot of solid, liquid or gaseous oxidizing agents. Preferably, it involves exposing the aliquot to a mixture of medical grade oxygen and ozone gas, most preferably by applying to the aliquot medical grade oxygen gas having ozone as a component therein. The ozone content of the gas stream and the flow rate of the gas stream are preferably selected such that the amount of ozone introduced to the blood aliquot, either on its own or in combination with one of the other stressors, does not give rise to excessive levels of cell damage, and so that, when the treated aliquot is injected into a subject, the desired effect will be achieved. Suitably, the gas stream has an ozone content of up to about 300 μg/ml, preferably up to about 100 μg/ml, more preferably about 30 μg/ml, even more preferably up to about 20 μg/ml, particularly preferably from about 10 μg/ml to about 20 μg/ml, and most preferably about 14.5 ± 1.0μ g/ml. The gas stream is suitably supplied to the aliquot at a rate of up to about 2.0 litres/min, preferably up to about 0.5 litres/min, more preferably up to about 0.4 litres/min, even more preferably up to about 0.33 litres/min, and most preferably about 0.24 ± 0.024 litres/min. The lower limit of the flow rate of the gas stream is

preferably not lower than 0.01 litres/min, more preferably not lower than 0.1 litres/min, and even more preferably not lower than 0.2 litres/min.

The ultraviolet light stressor is suitably applied by irradiating the aliquot under treatment from a source of UV light. Preferred UV sources are UV lamps emitting UV-C band wavelengths, i.e. at wavelengths shorter than about 280 nm. Ultraviolet light corresponding to standard UV-A (wavelengths from about 315 to about 400 nm) and UV-B (wavelengths from about 280 to about 315) sources can also be used. As in the case of the oxidative stressor, the UV dose should be selected, on its own or in combination of the other chosen stressor(s), so that excessive amounts of cell damage do not occur, and so that, when the treated aliquot is injected into a subject, the desired effect will be achieved. For example, an appropriate dosage of such UV light, can be obtained from up to eight lamps arranged to be exposed to the sample container holding the aliquot, operated at an intensity to deliver a total UV light energy at 253.7 nm at the surface of the blood of from about 0.025 to about 10 joules/cm^2, preferably from about 0.1 to about 3.0 joules/cm^2. Such a treatment, applied in combination with either the oxidative environment stressor or the temperature stressor, provides a modified blood aliquot which is ready for injection into the subject.

It is preferred to subject the aliquot to the oxidative environment stressor, the UV light stressor and the temperature stressor simultaneously, following the subjection of the aliquot to the mechanical stress, e.g. by extraction of the blood from the patient. Thus, the aliquot is preferably maintained at a predetermined temperature above or below body temperature while the

oxygen/ozone gas mixture is applied thereto and while it is irradiated with ultraviolet light.

The time for which the aliquot is subjected to the stressors is normally within the time range of up to about 60 minutes. The time depends to some extent upon the chosen combination of stressors. When UV light is used, the intensity of the UV light may affect the preferred time. The chosen temperature level may also affect the preferred time. When oxidative environment in the form of a gaseous mixture of oxygen and ozone applied to the aliquot is chosen as one of the two stressors, the concentration of the oxidizing agent and the rate at which it is supplied to the aliquot may affect the preferred temperature. Some experimentation to establish optimum times may be necessary on the part of the operator, once the other stressor levels have been set. Under most stressor conditions, preferred times will be in the approximate range of from about 2 to about 5 minutes, more preferably about 3 minutes. The starting blood temperature, and the rate at which it can be warmed or cooled to a predetermined temperature, tends to vary from subject to subject. Warming is suitably by use of one or more infrared lamps placed adjacent to the aliquot container. Other methods of warming can also be adopted.

As noted, it is preferred to subject the aliquot of blood to a mechanical stressor, as well as the chosen stressor(s) discussed above. Extraction of the blood aliquot from the patient through an injection needle constitutes the most convenient way of obtaining the aliquot for further extracorporeal treatment, and this extraction procedure imparts a suitable mechanical stress to the blood aliquot. The mechanical stressor may be supplemented by subsequent processing, for example the additional mechanical stress as the oxidative stressor is applied.

In the practice of the preferred process of the present invention, the blood aliquot may be treated with the heat, UV light and oxidative environment stressors using an apparatus of the type described in aforementioned U.S. Patent No. 4,968,483 to Mueller. The aliquot is placed in a suitable, sterile container, which is fitted into the machine. When UV is one of the chosen stressors, a UV-permeable container is used and the UV lamps are switched on for a fixed period before the other stressor is applied, to allow the output of the UV lamps to stabilize. When the stressor combination includes UV and temperature, the UV lamps are typically on while the temperature of the aliquot is adjusted to the predetermined value, e.g. 42.5 ± 1 °C. Four UV lamps are suitably used, placed around the container.

In the preferred method of the invention, a mammalian patient is given one or more courses of treatments, each course of treatment comprising the administration to a mammalian subject of one or more (e.g. one to six) aliquots of mammalian blood modified as discussed above.

For optimum effectiveness of the treatment, it is preferred that no more than one aliquot of modified blood be administered to the subject per day, in one or more injection sites, and that the maximum rest period between any two consecutive aliquots during the course of treatment be no greater than about 21 days. As used herein, the term "rest period" is defined as the number of days between consecutive aliquots or consecutive courses of treatment on which no aliquots of modified blood are administered to the subject.

Therefore, except where aliquots are administered to the subject on consecutive days, a rest period of from 1 to 21 days is provided between any two aliquots during the course of treatment. Moreover, at least one of the rest periods during the course of treatment preferably has a length of about 3 to 15 days.

Although it may be sufficient to administer only one course of treatment as described above to the subject, it may be preferred in some circumstances to administer more than one course of treatment, or to follow the above-described course of treatment by periodic "booster" treatments, if necessary, to maintain the desired effects of the present invention. For example, it may be preferred to administer booster treatments at intervals of 3 to 4 months following the initial course of treatment, or to administer a second course of treatments to the subject following a rest period of several weeks or months.

The invention is further illustrated and described with reference to the following specific example, comprising animal studies conducted in an approved manner.

EXAMPLE 1- Inflammatory Allergic Reactions associated with Contact Hypersensitivity

The effectiveness of the treatment according to a preferred embodiment of the present invention, on contact hypersensitivity (CHS), was assessed on laboratory mice, according to approved animal experimentation procedures, using the method described by Kondo et. al., "Lymphocyte function associated antigen-1 (LFA-1) is required for maximum elicitation of

allergic contact dematitis" Br J.Dermatol. 131:354-359, 1994, with minor variations.. The disclosure thereof is incorporated herein by reference. Briefly, to induce CHS, the abdominal skin of mice were shaved and painted dinitrodifluorobenzene DNFB, the sensitizing chemical, using 25 ul of DNFB in 4:1 acetone:olive oil solution. This sensitization was applied to four groups of five Balb C mice.

Whole blood was obtained from Balb C mice, by extraction from a main artery through an injection needle, and treated with an anti-coagulant. An aliquot of this was subjected to the process of a preferred embodiment of the invention, to obtain treated blood. The remainder was left untreated, for use in control experiments.

To obtain treated blood, the selected aliquot, in a sterile, UV-transmissive container, was treated simultaneously with a gaseous oxygen/ozone mixture and ultraviolet light at elevated temperature using an apparatus as generally described in aforementioned U.S.Patent No. 4,968,483 Mueller et.al. Specifically, 10 ml of citrated blood was transferred to a sterile, low density polyethylene vessel (more specifically, a Vasogen VC7002 Blood Container) for ex vivo treatment with stressors according to the invention. Using an apparatus as described in the aforementioned Mueller patent (more specifically, a Vasogen VC7001 apparatus), the blood was heated to 42.5 ± 1 °C and at that temperature irradiated with UV light principally at a wavelength of 253.7 nm, while oxygen/ozone gas mixture was bubbled through the blood to provide the oxidative environment and to facilitate exposure of the blood to UV. The constitution of the gas mixture was 14.5 ± 1.0 μg ozone/ml, with the remainder of the mixture comprising medical grade oxygen. The gas mixture was bubbled through the aliquot at a rate of 240 ± 24 ml/min for a period of 3 minutes.

Of the 4 groups of sensitized mice, the first, control group A received no treatment. The second, control group B, were treated with physiological saline, 50μl. The third, control group C, were sham treated, with 50μl of blood which had been extracted but not treated with the stressors. The fourth, test group D, were treated with 50μl of blood subjected to stressors as described above. Treatments, each involving intramuscular injection of 50 μl of the respective liquid, started on the day of sensitization, and was repeated every day for a total of 6 days. On the same day as the last treament, but after its administration, the animals were challenged with DNFB, by applying to the ears of each animal 10μl of 0.2% solution of DNFB. Inflammation due to CHS manifests itself in a swelling of the ears. Ear thickness was measured, 24 hours after challenge, with a Peacock spring-loaded micrometer (Ozaki Co., Tokyo, Japan). The results were expressed as the change (from pre-challenge level) in ear thickness and represent the mean maximal increase at 24 hours after challenge.

The experiments were repeated two more times, using two more sets of four groups of animals, to ensure statistical significance in the results. Figure 1 of the accompanying drawings is a graphical presentation of these results. A notable and significant reduction in ear thickness (inflammation) is to be observed with the animals treated according to this preferred process of the invention, as compared with any of the other groups. Figure 2 of the accompanying drawings represent photographs of cross-sections of the ears of a representative treated animal of group D (picture (a)) and a representative untreated group A animal (picture(b)). The decreased skin thickness, and the reduced lymphocyte infiltration (lower density of dark stained cells) is readily apparent on picture (a) from the treated animal, further demonstrating a significant

reduction in inflammation.

EXAMPLE 2 - Inflammation associated with Arthritis

The beneficial effects of the present invention on inflammation consequent upon arthritis have been demonstrated *in vivo* by clinical experiments on rats, specifically male Lewis rats in which rheumatoid arthritis had been induced.

Rheumatoid arthritis (RA) is characterized by a chronic inflammation of the synovial joints. An animal model used for studying RA is adjuvant induced arthritis in a rat model (see, for example, Pearson, C., 1956, "Development of Arthritis, periarthritis and periostitis in rats given adjuvant," *Proc. Soc. Exp. Biol. Med., 91:95*). According to this model, arthritis is induced in rats by injecting them with adjuvant containing *M. Butyricum.*

Male Lewis rats, 4-5 weeks of age, 100-120gms, were obtained from Charles River Laboratories, quarantined one week and entered into the study. An adjuvant mixture was prepared for induction of RA by suspending 50 mg *Mycobacterium butyricum* (Difco Laboratories, Inc., Detroit, Michigan) in 5ml light white paraffin oil - m3516 (Sigma Chemical Co., St Louis, MO)- and thoroughly mixed using a homogenizer. Aliquots of the mixture sufficient to supply 0.15 mg *M. Butyricum* was injected into each animal subcutaneously, at the base of the tail. Inflammation due to RA started about 12 days after induction, in each animal, evidenced by limb swelling.

On each treatment day, two of the injected rats were used as blood donors. Blood was collected from them by cardiac puncture, and 10 ml of citrated blood was transferred to a sterile,

open, low density polyethylene vessel (more specifically, a Vasogen VC7002 Blood Container) for ex vivo treatment with stressors according to the invention. Using an apparatus as described in the aforementioned Mueller patent (more specifically, a Vasogen VC7001 apparatus), the blood was heated to 42.5±1°C and at that temperature irradiated with UV light at a wavelength of 253.7 nm, while oxygen gas was bubbled through the blood to facilitate exposure of the blood to UV, but without use of the oxidatively reactive ozone gas, for 3 minutes.

14 rats were given a course of 10 injections of 0.2 ml aliquots of the treated blood, every second day for 20 days. A control group of 10 rats received a similar course of injections with saline. Injections commenced one day after the induction of RA. Hind paw volumes of the animals were measured as a measure of inflammation indicative of RA, on alternate days, after onset of RA, by water displacement in a 250 ml beaker using a top-loaded Mettler balance. The respective results were averaged over the two separate groups of animals, experimental and control, and the results are presented graphically on the accompanying Figure 3, a plot of mean hind paw volume against days after RA induction. The upper curve (round points) is derived from the control group of animals which received saline, the lower curve (square points) from the animals which received the course of injections of treated blood.

A significant decrease in the severity of the RA, as indicated by lower foot volumes, is apparent for the treated animals as compared to the animals of the control group.

EXAMPLE 3 - Inflammation associated with atherosclerosis

Model:

This experiment demonstrates the effects of treatment according the present invention on the development of atherosclerosis and associated vascular wall inflammation in the LDL receptor (LDL-R) deficient mouse model, a widely used transgenic atherosclerosis model created by targeted disruption of the LDL receptor. This animal model is analogous to familial hypercholesterolemia, an inherited condition in which a mutation results in complete lack of functional LDL-R. In the human disease, homozygous individuals demonstrate a marked increase in serum cholesterol and develop severe premature atherosclerosis, with associated vascular inflammation, often succumbing to this disease at an early age.

The LDL-R deficient mouse model shows intolerance to cholesterol feeding and develops widespread atherosclerotic changes which progress to mature fibrous lesions morphologically indistinguishable from established human atherosclerosis. Apart from the defined genetic abnormality causing predisposition to atherosclerosis, this model has the advantage of rapid development of widespread atherosclerosis within 6 to 8 weeks following institution of cholesterol feeding.

Protocol:

LDL-R deficient mice were purchased from Jackson Laboratories. A total of 20 mice were entered into the study at 22 weeks of age, and 15 mice completed the study. The length of the study was 8 weeks. The mice were maintained on a 12 hour dark/12 hour light cycle with free access to food and water, and were fed a specified diet as follows. A control group comprising 5 animals, all of which completed the study, received a normal diet. The high

cholesterol group comprising 15 animals, of which 10 completed the study, were fed a diet containing 1.25% cholesterol, 7.5% cocoa butter, 7.5% casein, and 0.5% sodium cholate. To ensure proper food intake, food consumption and animal weight were monitored on a weekly basis. In previous experiments, it was demonstrated that 8 weeks of feeding with the high cholesterol diet results in substantial atherosclerosis development, particularly in the aortic arch and the descending thoracic aorta.

Treatment:

Ten of the animals fed the high cholesterol diet were selected at random to undergo a course of treatment by the preferred method of the invention. Six of the treated animals completed the study. It is to be noted that the four deaths in this group were not in any way related to the treatment, but occurred early in the study as a result of fighting among animals which were housed together during the study. The other five animals on the high cholesterol diet underwent a course of sham treatments with untreated blood, and four survived the protocol.

The treatments began four weeks after initiation of the study, with each of the animals on the high cholesterol diet receiving a total of 10 treatments (2 courses of treatment of 1 injection per day for 5 days, the 2 courses of treatment separated by two days, i.e. 10 injections over a period of 12 days). Each individual treatment administered to the animals treated by the method of the present invention consisted of the collection of 10 ml of blood from genetically compatible donor animals fed on a normal diet, the blood being collected into sodium citrate anticoagulant. In order to collect each 10 ml aliquot of blood, about 1 ml of blood was extracted from each of

10 animals. The blood was extracted by cardiac puncture, with the animals being under full xylazine/ketamine anesthesia during the blood extraction procedure, and being given T-61 immediately following extraction. The blood aliquot was transferred to a sterile, disposable, low-density polyethylene vessel for ex vivo treatment, and was then treated simultaneously with a gaseous oxygen/ozone mixture and ultraviolet light at elevated temperature using an apparatus as generally described in aforementioned U.S. Patent No. 4,968,483 to Mueller et al.

The constitution of the gas mixture was 14.5 ± 1.0 μg ozone/ml, with the remainder of the mixture comprising medical grade oxygen. The gas mixture was bubbled through the aliquot at a rate of 240 ± 24 ml/min for a period of 3 minutes. The temperature of the aliquot was held steady at 42.5 ± 1.0°C. The UV light was within the UV-C band, and included a wavelength of 253.7 nm.

After treatment by the preferred method of the present invention, 30 μl of the treated blood was re-injected intramuscularly into each animal undergoing treatment according to the present invention.

In the sham treatments, 30 μl of untreated blood was injected intramuscularly into each of the remaining five animals on the high cholesterol diet.

Assessment of Atherosclerosis:

After 8 weeks, the animals were anesthetized with zylaxine/ketamine and the heart was

exposed. After nicking the vena cava to obtain blood samples, the animals were perfused via ventricular puncture, first with PBS to flush out the blood and then with 10% neutral buffered formalin for 3 minutes to fix the aorta. The thoracic aorta was dissected away from the thorax en bloc and stored in 10% formalin at 4°C. Pressure-fixed (10% formalin) aortae were removed en bloc and opened to allow a longitudinal full length inversion. The aortae were then mounted internally exposed on glass slides and stained with oil red O. The bright red staining (indicating lipid deposition) was then quantified using a computer assisted morphometric system, and expressed as a percentage of total aortic intimal surface.

Statistical Analysis:

Continuous variables are reported as mean ± SD. Differences in cholesterol levels and triglyceride levels among groups were tested by Student's t-test. Differences in atherosclerotic lesion area among groups were tested using the one-way ANOVA test in conjunction with the Bonferroni correction.

Figure 4 illustrates two full length aorta stained with oil red O to detect lipid deposition and plaque formation inside the arteries. The animals which received the high cholesterol diet and the sham treatments exhibited substantial aortic lipid deposition (aorta "A" in Fig. 4), with a ratio of atherosclerotic area (AA) to total area (TA) being 0.16±0.1. This is accompanied by significant vascular inflammation. In comparison, those animals which were treated by the preferred method of the invention showed a profoundly reduced level of aortic lipid deposition (aorta"B" in Fig 4), with AA/TA being 0.04±0.03. These ratios are significantly different, with

$p<0.05$. In the animals which received the normal diet, no significant atherosclerotic changes were observed.

In addition, the animals which were treated according to the preferred method of the invention were observed to have better general appearance, reduced skin xanthomatosis (eyelids, nose and paws), reduced limb swelling indicative of significantly reduced inflammation, and better appetite than the untreated animals which received the high cholesterol diet.

Although the invention has been described in connection with certain preferred embodiments, it is to be appreciated that it is not limited thereto. Rather, the present invention includes within its scope all embodiments which may fall within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method of treatment or prophylaxis of chronic or acute inflammatory and/or allergic disorders in mammalian subjects, which comprises administering modified mammalian blood to the subject, the blood having been modified extracorporeally by simultaneous or sequential exposure to at least one stressor selected from the group consisting of an oxidative environment, UV radiation, and temperature above, at or below body temperature,.

2. The method of claim 1 wherein the stressors are a combination of oxidative environment and temperature above, at or below body temperature.

3. The method of claim 1 wherein the stressors are a combination of oxidative environment and UV radiation.

4. The method of claim 1 wherein the stressors are a combination of UV radiation and a temperature above, at or below body temperature.

5. The method of claim 1 wherein the stressors are UV radiation, temperature above body temperature and optionally an oxidative environment, supplied substantially simultaneously to the blood aliquot.

6. The method of claim 1, claim 2, claim 3, claim 4 or claim 5, further including the application of mechanical stress to the aliquot.

7. The method of claim 6 wherein the oxidative environment is a gaseous mixture of oxygen and ozone, bubbled through the aliquot.

8. The method of claim 6 wherein the temperature stressor is a temperature in the approximate range 37-55°C.

9. The method of any of claims 1-8 wherein the inflammatory and/or allergic disorder is a dermatological disorder associated with contact or delayed type hypersensitivity.

10. The method of claim 9 wherein the disorder is contact hypersensitivity.

11. The method of claim 9 wherein the disorder is delayed type hypersensitivity.

12. The method of claim 9 wherein the disorder is chronic or acute contact dermatitis.

13. The method of claim 9 wherein the disorder is atopic dermatitis.

14. The method of claim 9 wherein the disorder is acute or chronic urticaria

15. The method of claim 9 wherein the disorder is eczema.

16. The method of claim 9 wherein the inflammatory disorder is a skin contact allergic reaction.

17. The method of any of claims 1-8 wherein the disorder is inflammation due to arthritis.

18. The method of any of claims 1-8 wherein the disorder is vascular inflammation associated with atherosclerosis.

19. The method of any of claims 1-8 wherein the disorder is inflammatory allergic vasculitis.

20. The method of any of claims 1-8 wherein the disorder is a respiratory disorder involving inflammation of breathing tubes.

21. The method of claim 20 wherein the disorder is asthma.

22. The method of claim 20 wherein the disorder is hay fever.

23. The method of any of claims 1-8 wherein the disorder is a food or drug inflammatory or allergic reaction.

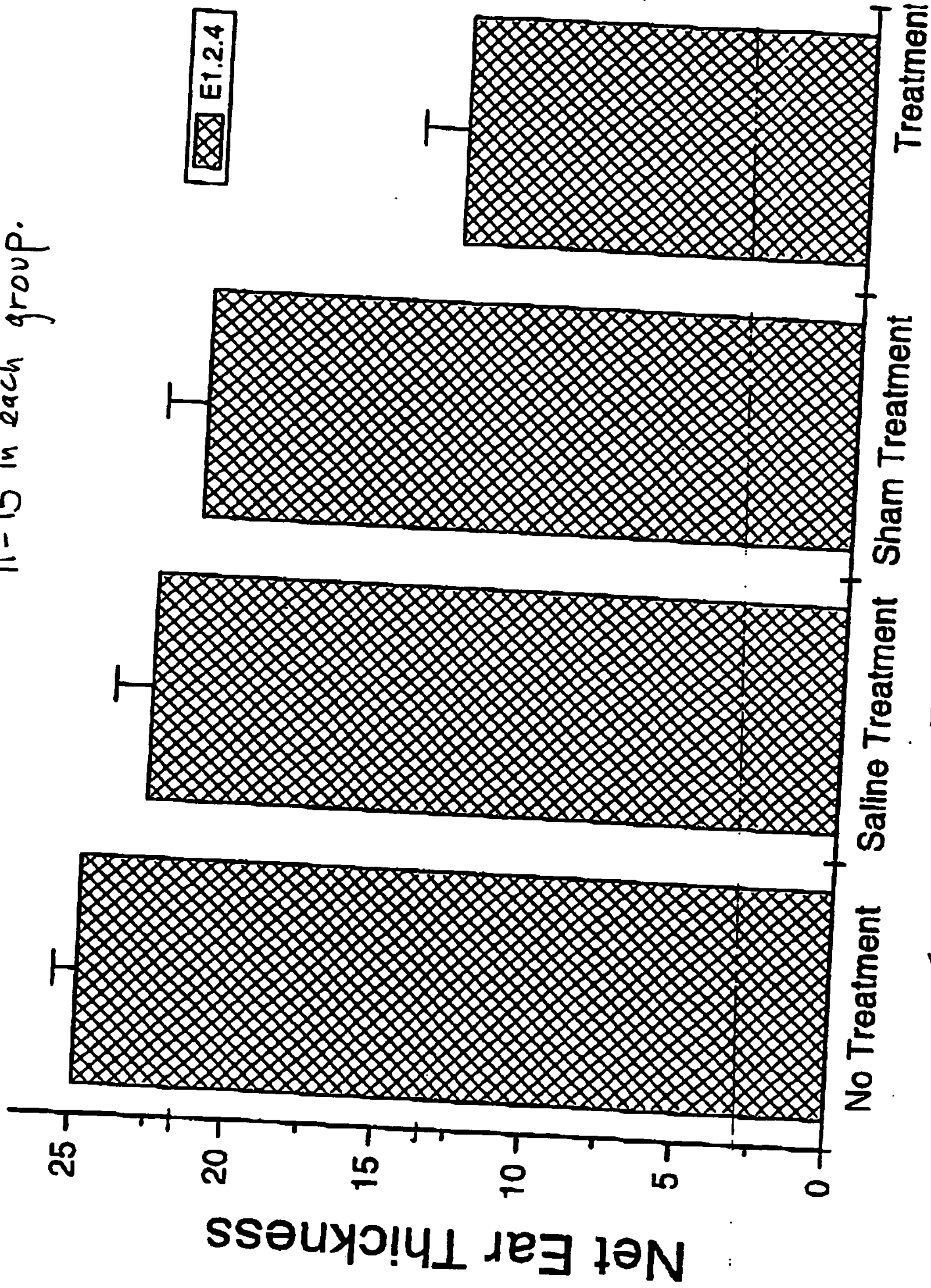
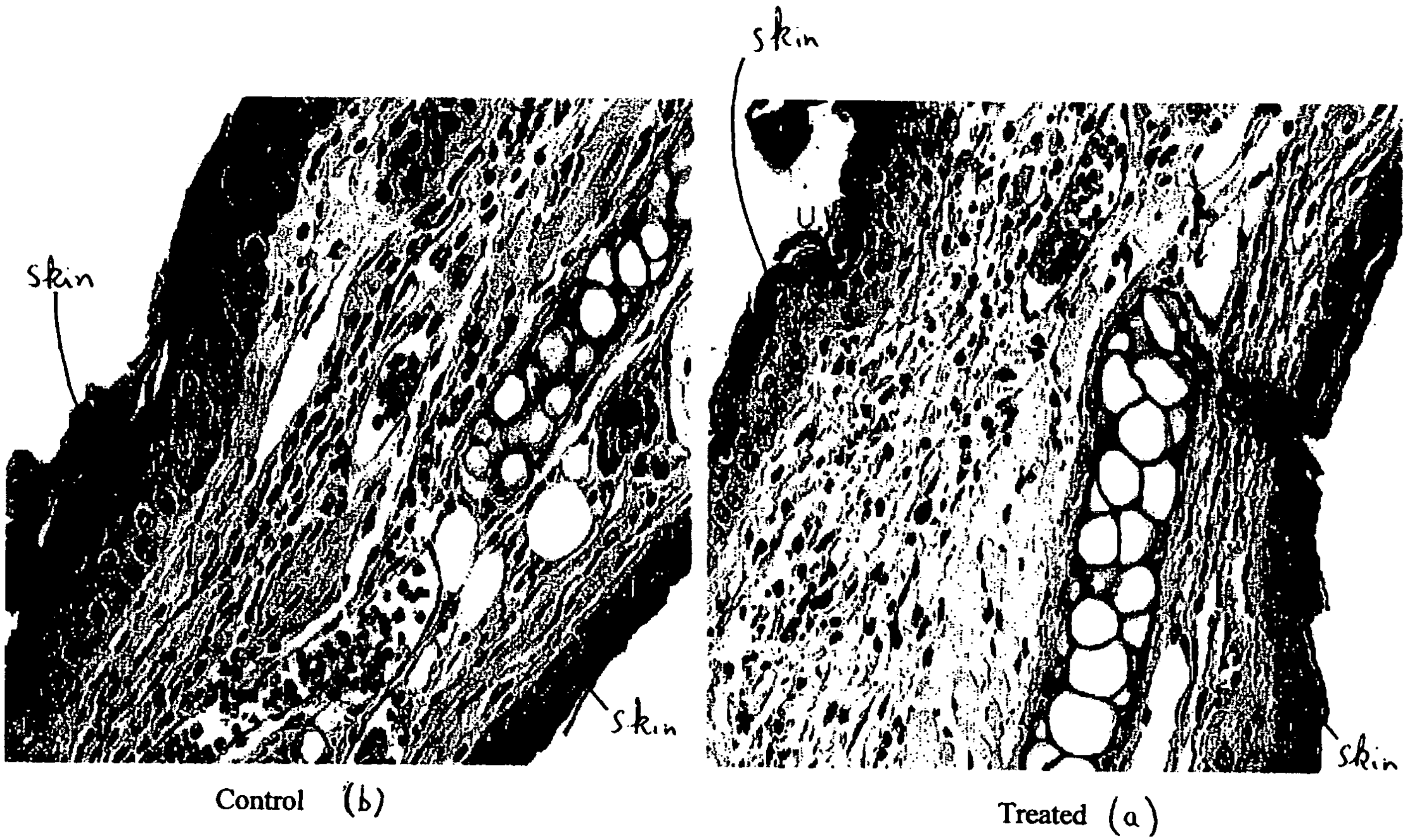


Fig. 1



Control (b)

Treated (a)

FIG. 2

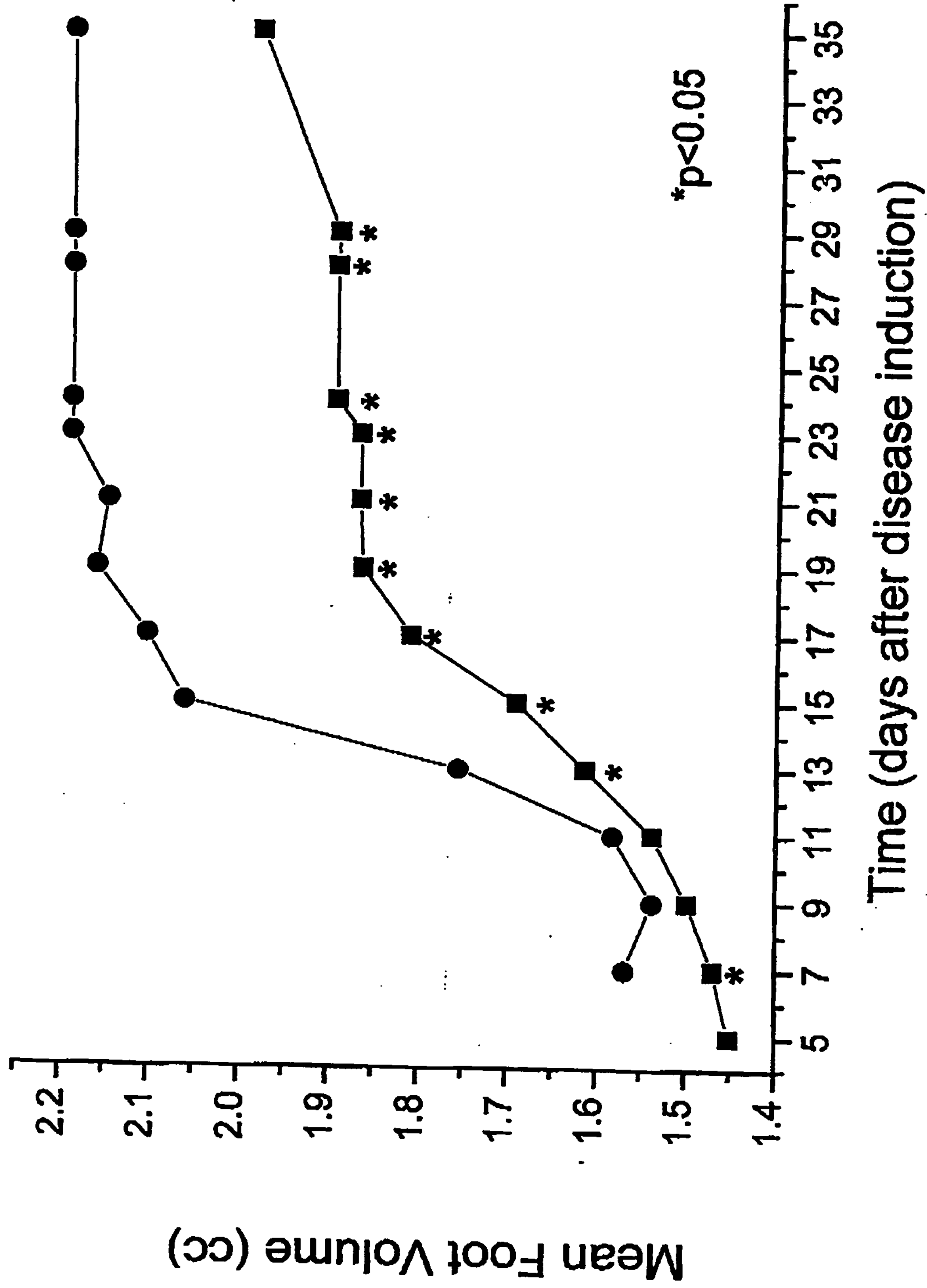


FIG. 3

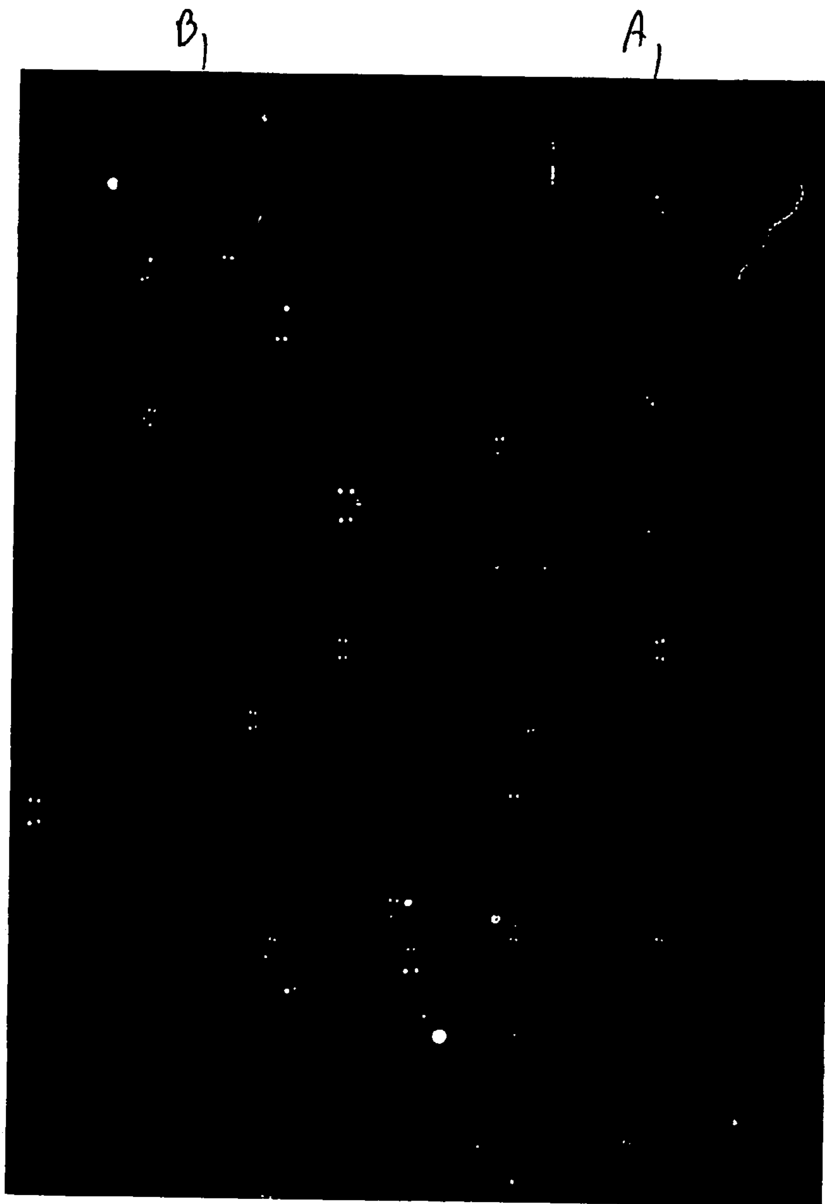
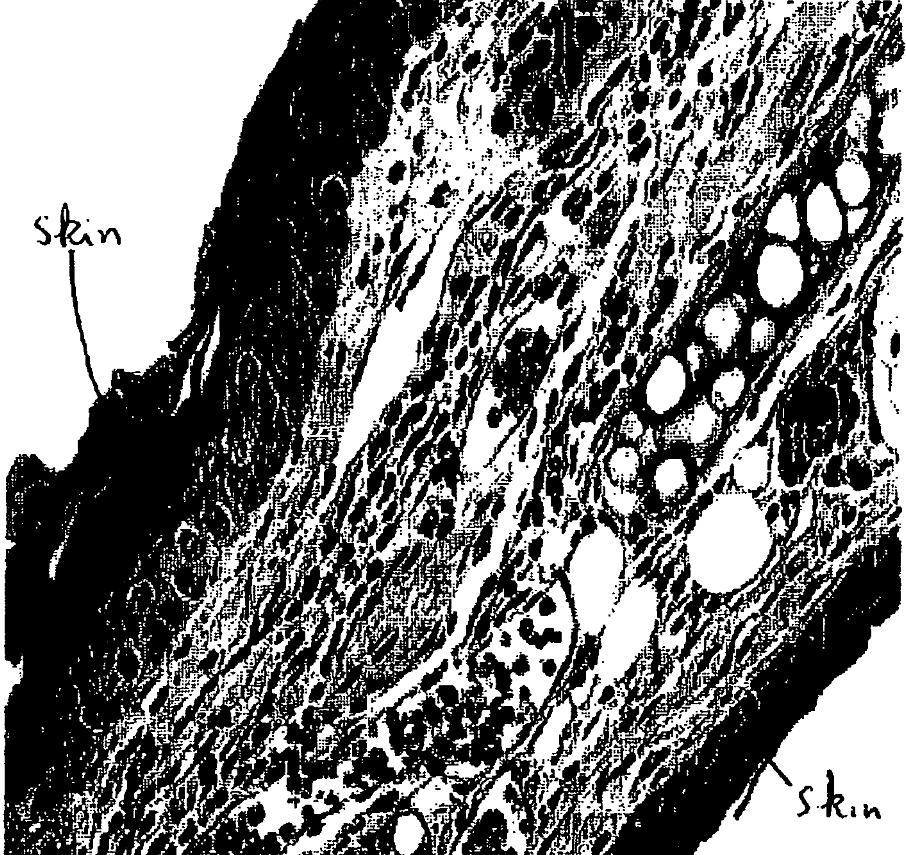
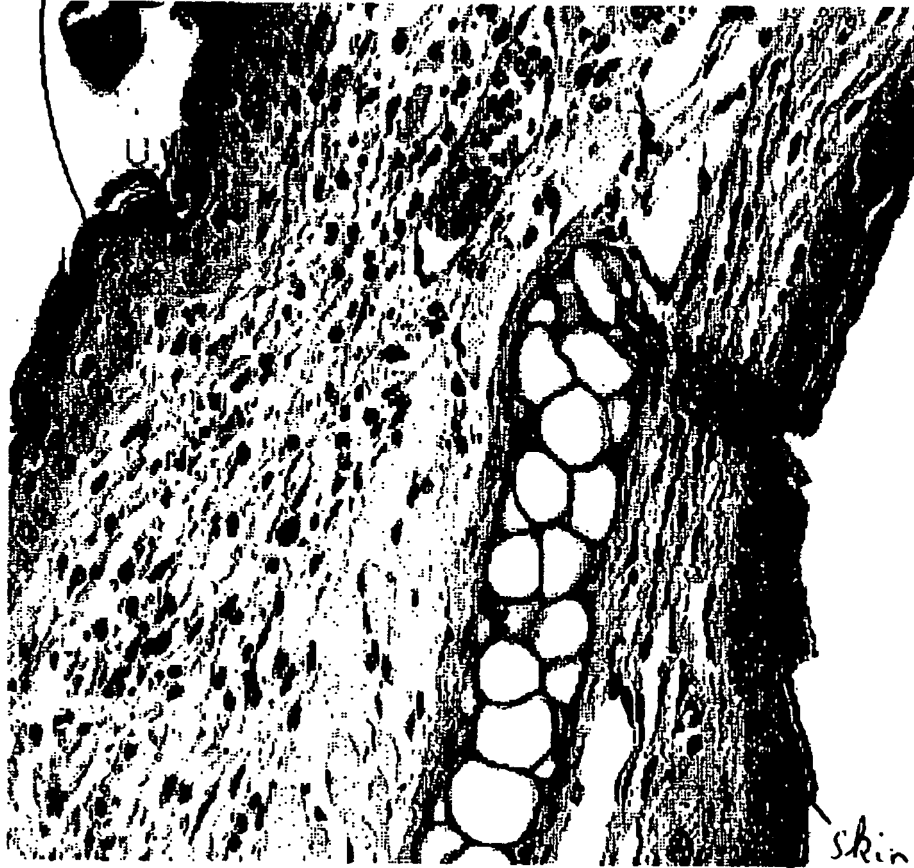


FIG. 4



Control (b)



Treated (a)